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14. ABSTRACT: The aims of this project are to determine the phenotype and antileukemic activity of activated bone marrow infiltrating leukemia (MIL) and compare them to activated peripheral blood lymphocytes from patients with chronic myelogenous leukemia (CML) on imatinib or other tyrosine kinase inhibitor therapy. Bone marrow and peripheral blood specimens were obtained from CML patients who had at least a minor cytogenetic response. The phenotype of MILs and peripheral blood lymphocytes (PBL) was analyzed by flow cytometry. MILs and PBLs were expanded and activated with anti CD3/CD28 magnetic beads in culture for 10 days. Activated MILs and PBLs were characterized by flow cytometry and tested for antileukemic activity in a colony suppression assay by coculture with CD34+ bone marrow progenitors at varying CD3:CD34 ratios in methycullulose medium. Analysis of the phenotype of MILs from four patients showed that MILs are predominantly comprised of effector memory T cells. These MILs could be expanded effectively without change in phenotype. MILs as well as activated PBLs showed ability to suppress CML progenitor growth in vitro.					
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DOD final report

Introduction

Imatinib and other tyrosine kinase inhibitors, although highly effective drugs for treatment of CML are not curative and relapse occurs quickly after their discontinuation. Since the leukemic cells in CML express various tumor antigens that are recognized by the immune system, generation of an effective immune response has the potential to eradicate leukemia and achieve cure. Relapse of CML can occur after allogeneic HCT, necessitating the use of donor lymphocyte infusions with its attendant toxicity, especially induction of severe GVHD. Thus better methods of augmenting allogeneic and autologous immune response against CML are needed. Bone marrow infiltrating lymphocytes (MILs) have been studied in tumors like breast cancer and have been shown to be comprised of activated central and effector memory T-cells with a restricted T cell receptor repertoire suggesting that these cells represent a specific immune response. No data exists on the phenotype or function of MILs in patients with CML. This study was aimed to characterize the phenotype MILs in CML as well as to study the ability of these cells to suppress leukemic progenitor cell proliferation. We also evaluated the ability to expand MILs for potential therapeutic application in future clinical trials.

Study Results

Bone marrow and peripheral blood samples from patients who had at least a minor cytogenetic response to imatinib or nilotinib and in whom a diagnosis bone marrow specimen was available were studied. Two patients were in accelerated phase and 4 patients were in chronic phase. We were unable to study patients who had undergone allogeneic HSCT since this procedure is rarely performed nowadays with the advent of tyrosine kinase inhibitors.

Task 1. Characterize the immunophenotype, T cell receptor VB repertoire of MILs.

Methods: Bone marrow mononuclear cells (BMNC) or peripheral blood mononuclear cells (PBMC) was obtained by density gradient centrifugation of bone marrow aspirate or peripheral blood respectively. BMNC and PBMC were stained with fluorochrome-conjugated antibodies to CD3, CD4, CD8, CD45RA, CD45RO, CCR7, CD62L, CXCR4, CD25, and IFN- γ (BD PharMingen, SanDiego, CA) and analyzed in a multicolor Beckman Coulter FC500 flow cytometer using FCS Express software (Beckman Coulter, Fullerton, CA). In the CD4 and CD8 T cell subsets, naïve T cells were defined by their coexpression of CD45RA and CD62L. Effector T cells are CD45RA⁺, CD62L⁻. Memory cells were defined by their expression of CD45RO and absence of CD45RA. The central memory subset expresses CCR7 and CD62L while the effector memory subset was defined as CCR7⁻, CD62L⁻. Regulatory T cells were defined by their coexpression of CD4 and CD25.

Results

CD8 T- cells comprised an average of 6 % (SD 6.6%) of (BMNC) and 8.4 % (SD 8%) of PBMC. CD4 T-cells comprised 13.5 (SD 13.8) % of BMNC and 22.7% (SD 20.7) of PBMC. The percentages of B-cells (CD19+), NK cells (CD56+) and total T-cells (CD3+) is shown in Supplemental Table 1.

CD4 and CD8 cells in the bone marrow and peripheral blood were subtyped into naïve and memory subsets based on their expression of CD45RA and CCR7 as described above. In the bone marrow effector memory T-cells (CCR7-, CD45RA-) comprised a mean of 93.3% of CD4 T-cells and 82.7% of CD8 T cells. The percentages of all lymphocyte subsets in the bone marrow and peripheral blood are shown in Supplemental Table 2. Therefore it can be concluded that effector memory T cells are the most abundant subset of bone marrow T-cells similar to peripheral blood. VB repertoire of MILs did not show any skewing.

Task 2. Expand MILs and determine the phenotype, T cell receptor VB repertoire of expanded MILs.

Methods: BMNCs or PBMC were combined with anti-CD3/anti-CD28 beads (Dynal) at 3:1 bead-to-T cell ratios in AIM-V medium with 5% heat-inactivated human serum and 100 IU/mL IL-2 and cultured for 7-10 days in 24 well plates. The expanded MILs and PBLs were analyzed by flow cytometry as described under Task 1 and also used for colony suppression assays described below under Task 3.

Results

Expansion of bone marrow and peripheral blood T cells showed marked inter patient variability and ranged from 5 to 15 fold for peripheral blood and 5 to 35 fold for bone marrow. The phenotype of the expanded cells showed mean of 75% T cells (bone marrow) and 74% T cells for peripheral blood. CD8 cells comprised 20.2% (SD 16%) and CD4 cells comprised 27% (SD 15%) of expanded bone marrow T cells while the CD8 and CD4 percentages for expanded peripheral blood lymphocytes (PBL) were 27% (SD 14.4) and 26% (SD 21) respectively. Subset analysis of CD4 and CD8 T cells again showed a preponderance of effector memory CD4 and CD8 T cells similar to the preexpansion specimens (Supplemental Table 2 and Supplemental Figure 1). The expanded MILs and PBLs had high expression of CXCR4 suggestive of their potential to home to the bone marrow. (Figure 2)VB analysis of expanded MILs and PBLs failed to reveal any skewing. The absolute cell counts and subset analysis is shown in Supplemental Tables 1 and 2.

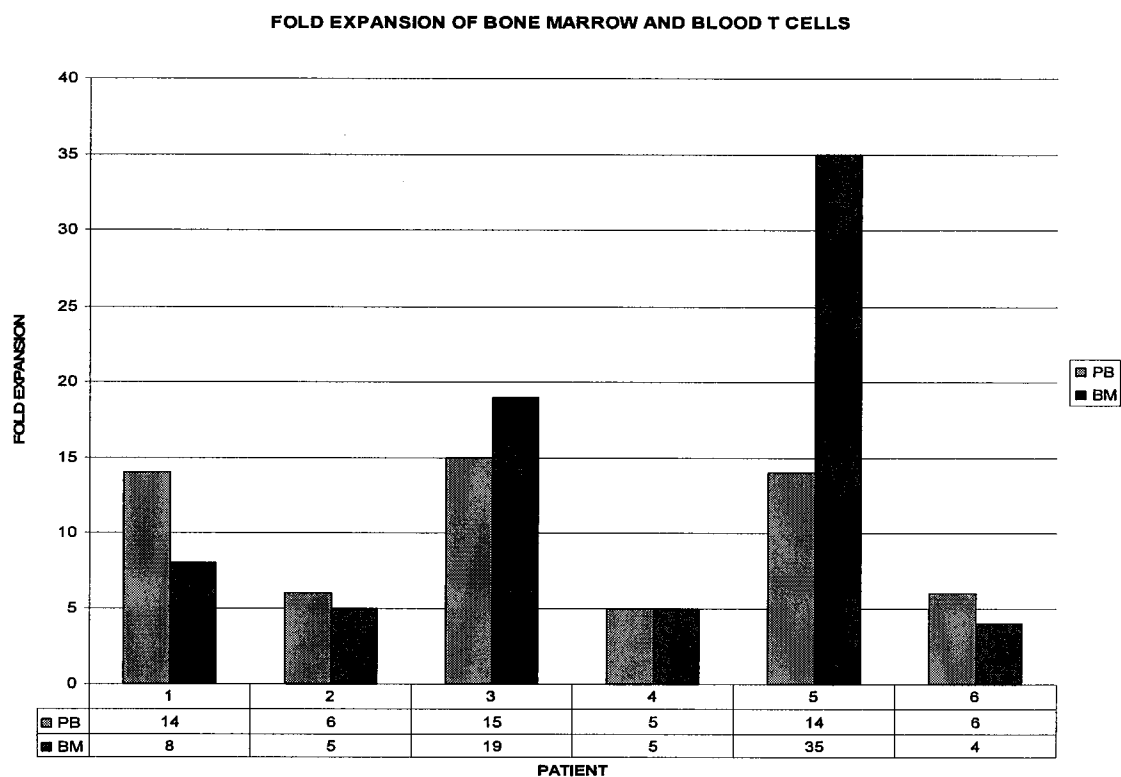


Figure 1. Expansion of peripheral blood (PB) and bone marrow (BM) T cells using CD3/CD38 magnetic beads

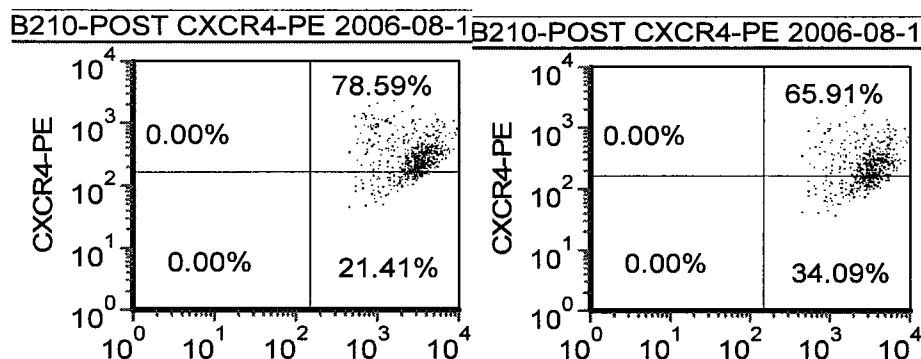


Figure 2. Phenotype of expanded MILs shows high expression of CXCR4 in the total T cells (left panel) as well as in effector memory cells (right panel) from a representative patient

Task 3. Determine antileukemic activity of expanded and activated MILs

Methods: BMNC obtained at diagnosis were cocultured with expanded MILs at varying effector: target ratios for 24 to 72 hours, in the presence of 20 IU/mL of IL-2 and low levels of growth factors (SCF and GM-CSF). Cells were washed and replated in methylcellulose for progenitor assays in presence of EPO, IL-3, SCF, G-CSF and GM-CSF for 14-18 days and assessed for CFU-GM and BFU-E colonies. CFC growth from BMNC cocultured with MILs were compared with controls incubated with PBL, and the percentage suppression in progenitor growth was calculated.

Cytotoxicity assay was performed in 2 patients by incubating Ph + CD34+ progenitors isolated from diagnosis specimen with expanded MILs or PBLs overnight. Degranulated CD8 cells that recognized leukemic cells were identified by surface staining with CD8 and CD107 antibodies.

Results

Both expanded MILs and PBLs were capable of suppressing growth of CML progenitors in a dose dependednt fashion in 4 of 5 patients tested, No significant difference in the percentage of growth inhibition was noted between expanded MILs and PBLs. The More pronounced growth suppression was noted for patients in chronic phase compared to patients in accelerated phase. The percentage of normal versus PH+ cells in the colonies varied depending on disease phase with normal cells predominating in patients in chronic phase as opposed to predominance of Ph+ cells in patients with accelerated phase disease. The results of colony suppression assays for 5 patients are shown in Supplemental Figure 2 and FISH results are shown in Supplemental Figure 3.

Cytotoxicity of expanded bone marrow or peripheral blood CD8 cells against CD34+ CML progenitors was not seen in the 2 patients tested (data not shown). Data from 2 representative patients is shown below.

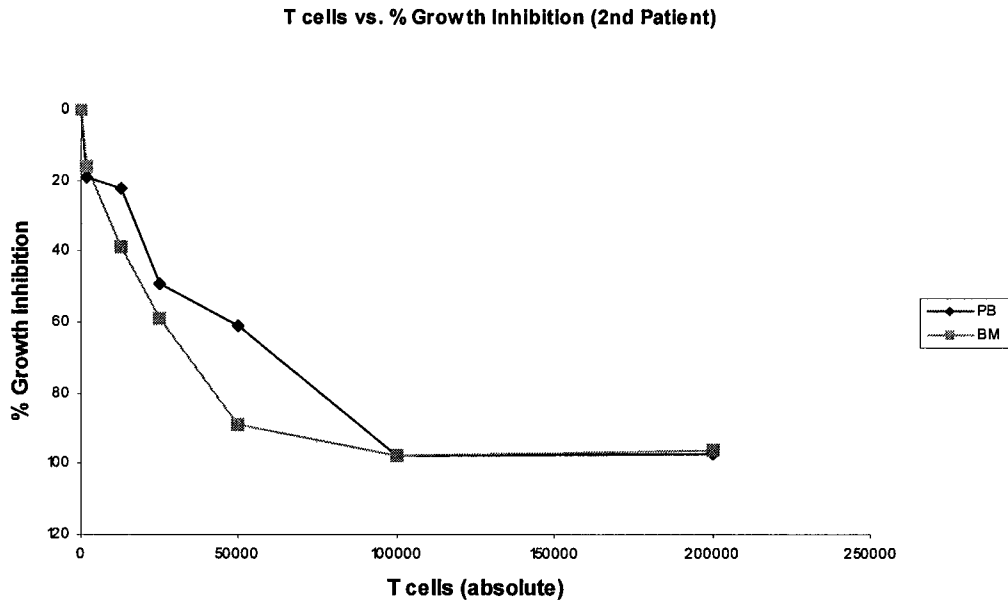
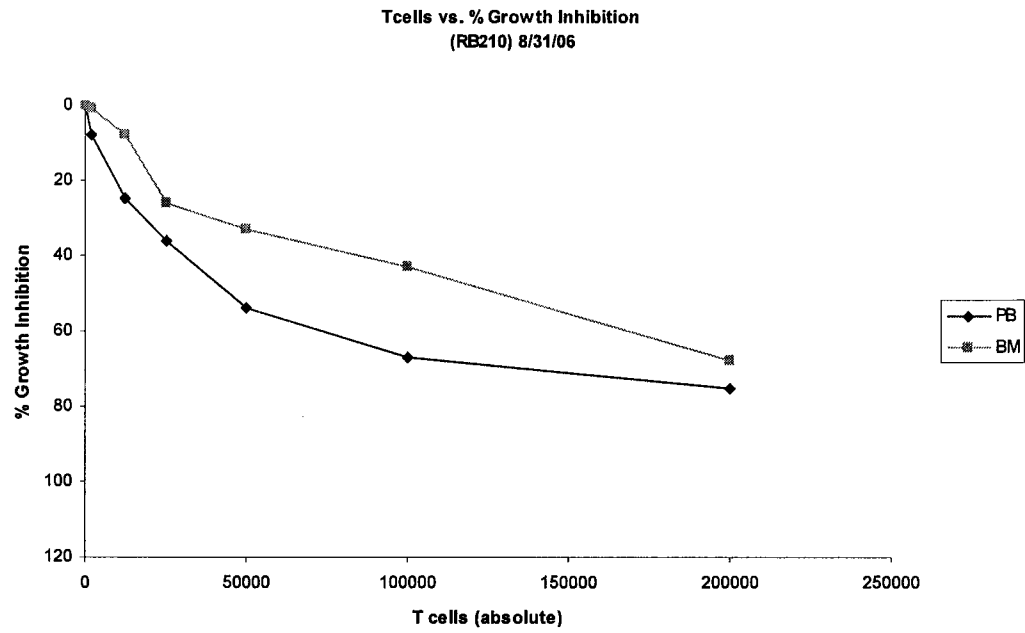


Figure 3. Dose dependent suppression of CML progenitor growth in culture is shown for expanded MILs (BM) and PBLs (PB) for two patients with chronic phase CML.

Key Research accomplishments

- We have determined that memory T cells are the predominant T cell population in the bone marrow of patients with CML who are on tyrosine kinase inhibitor therapy
- Bone marrow-derived and peripheral blood derived T-cells can be effectively expanded in some patients with CD3/CD28 magnetic beads without alteration of their phenotype
- Expanded bone marrow and peripheral blood T cells are capable of suppressing the growth of leukemic progenitors in vitro and may have potential for cellular immunotherapy.

Reportable Outcomes

Pullarkat V, Cuya S, Koenig H, Bhatia R. Bone marrow and peripheral blood T cells expanded with CD3/CD28 magnetic beads are capable of suppressing CML progenitor cell growth. Manuscript in preparation for submission to *Cancer Immunology and Immunotherapy*

Conclusion

The bone marrow of CML patients on tyrosine kinase inhibitors predominantly contains effector memory subset of CD4 and CD8 T cells. These cells can be expanded with CD3/CD28 magnetic beads without change in phenotype. These cells have high expression of the bone marrow homing receptor CXCR4 and would be expected to home to the bone marrow after intravenous administration. These expanded T cells were capable of suppressing the growth of CML progenitors in a dose dependent manner. These activated bone marrow or peripheral blood T cells could have therapeutic implications as a cell based vaccine for eradication of minimal residual disease in CML

Several aspects of this needs to project however needs to be further developed. We were unable to demonstrate that bone marrow was enriched in CML-specific cells and CD8 dependent cytotoxicity towards CML progenitors could not be demonstrated. This suggests that CML-specific CD4 or CD8 cells are infrequent in the marrow and need to be expanded specifically. We were limited by the quantity of bone marrow and peripheral blood collected at diagnosis. We are collecting samples from newly diagnosed patients and generating monocyte derived dendritic cells from these diagnosis blood specimens in an attempt to use these dendritic cells to selectively expand leukemia specific T cells from blood and bone marrow. We are also evaluating the ability of anti CTLA-4 antibodies to augment expansion of bone marrow and peripheral blood T cells.

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B. Education

College:	St. Joseph's College, University of Calicut Kerala, India, 1983-1985.
Medical School:	Calicut Medical College, University of Calicut, Calicut, Kerala, India, MB, BS Degree, 1985-1991.
Internship:	Rotating Internship, Calicut Medical College, India, 1991-1992.
Residencies:	Internal Medicine, Calicut Medical College, India, 1992-1994. Internal Medicine, New York Methodist Hospital, New York, 1994-1996
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Fellowships:	Hematology and Oncology, University of Southern California, Los Angeles, 1997- 2000. Bone Marrow Transplantation, 2002- 2003, City of Hope Medical Center, California

Honors and Awards:	First Rank in Pathology, Microbiology and Immunology, University of Calicut, India 1987
Licensure:	California, 1997 (#A063417)
Board Certification:	American Board of Internal Medicine, 1997
	Royal Colleges of Physicians (UK), 1997
	American Board of Hematology, 2000.
	American Board of Medical Oncology, 2000.

C. Professional Background

Academic appointments

- Clinical Instructor in Medicine, Division of Hematology, University of Southern California School of Medicine and Attending Physician Los Angeles County-USC Medical Center, Los Angeles. 2000-2002
- Clinical Instructor, City of Hope Medical Center, Duarte, California. 2002-2003
- Assistant Professor, Division of Hematology and Hematopoietic Cell Transplantation, City of Hope Medical Center, Duarte, California. 2003-Present

D. Society Memberships

Member, Royal Colleges of Physicians of the United Kingdom.
 Member, European Hematology Association
 Member, American Society of Hematology
 Member, American Society of Blood and Marrow Transplantation

E. Reviewer

Blood
 American Journal of Hematology
 Journal of Immunologic Methods
 Journal of Clinical Pathology
 Biology of Blood and Marrow Transplantation

F. Research Activities

Research Interests

Hematopoietic Stem Cell Transplantation

Acute leukemia
Autoimmune hematologic disease

Research Grants

1. US Department of Defense. Phenotype and function of marrow infiltrating lymphocytes in chronic myeloid leukemia. Principal Investigator. 2005-2007. \$ 150,000.

Current Protocols

1. City of Hope Protocol # 03162. Molecular pathogenesis of acute leukemia and myelodysplasia. (Principal Investigator)
2. City of Hope Protocol # 04040. Effect of non-HLA gene polymorphisms on the outcome of allogeneic stem cell transplantation. (Principal Investigator).
3. City of Hope Protocol #05199. An Open Label Study Evaluating the Safety and Efficacy of Long-Term Dosing of AMG 531 in Thrombocytopenic Subject with Immune (Idiopathic Thrombocytopenic Purpura (ITP). (Principal Investigator).
4. City of Hope Protocol #05095. A pilot Phase II trial of a synthetic tumor-specific breakpoint peptide vaccine in patients with chronic myeloid leukemia and minimal residual disease. (Principal Investigator).
5. City of Hope Protocol # 06149. A Randomized, Double-Blind, Placebo-Controlled Phase III Study, To Evaluate The Efficacy, Safety And Tolerability Of Eltrombopagolamine (SB-497115-GR), A Thrombopoietin Receptor Agonist, Administered For 6 Months As Oral Tablets Once Daily In Adult Subjects With Previously Treated Chronic Idiopathic Thrombocytopenic Purpura (ITP)
6. City of Hope Protocol # 06159. A Randomized, Double-Blind, Placebo-Controlled Study To Assess The Efficacy And Safety Of Prophylactic Use Of Maribavir For The Prevention Of Cytomegalovirus Disease In Recipients Of Allogeneic Stem Cell Transplants. (Principal Investigator).
7. City of Hope Protocol #5050. An Open Label, Safety and Tolerability Study of Deferasirox for Treatment of Transfusional Iron Overload in Low-risk and INT-1 Myelodysplastic Patients using Serum Ferritin Monitoring. (Principal Investigator).
8. City of Hope Protocol # 04065. Effect of differentiation status and tissue specificity of graft T cells on graft-versus-host disease following allogeneic peripheral blood stem cell transplantation. (Co-Investigator).
9. City of Hope Protocol # 04094. A hemoglobin stabilization and transfusion reduction efficacy and safety clinical investigation, randomized, multicenter

double-blind, placebo-controlled study using eculizumab in paroxysmal nocturnal hemoglobinuria. (Co-Investigator).

Bibliography.

Peer Review

1. **Pullarkat VA**, Slovak ML, Kopecky KJ et al. Impact of cytogenetics on the outcome of adult acute lymphoblastic leukemia: results of the Southwest Oncology Group study SWOG9400. *Blood* 2007; 111: 2563-2572.
2. Winston DJ, Young J-AH, **Pullarkat V** et al. Maribavir prophylaxis for prevention of cytomegalovirus infection in allogeneic stem-cell transplant recipients: a multicenter, randomized, double-blind, placebo-controlled, dose-ranging study. *Blood* (Epub ahead of print)
3. Bedell V, Forman SJ, Gaal K, **Pullarkat V**, Weiss LM, Slovak ML. Successful application of a direct detection slide-based sequential phenotype/genotype assay using archived bone marrow smears and paraffin embedded tissue sections. *Journal of Molecular Diagnostics*. 2007; 9: 1-9.
4. Kim Y, Weiss LM, Chen Y-Y,, **Pullarkat V**. Distinct Clonal Origins of Systemic Mastocytosis and Associated B-Cell Lymphoma. *Leukemia Research* 2007; 31: 1749-1754.
5. Kuter DJ, Bussel JB, Lyons RM, **Pullarkat V** et al. Randomized, controlled, 6-month evaluation of AMG 531 in patients with chronic immune thrombocytopenic purpura. *Lancet* 2008; 371: 395-403.
6. Nakamura R, Rodriguez R, Palmer J, Stein A, Naing A, Ttsai N, Chang K, Slovak ML, Bhatia R, Spielberger R, Kogut N, **Pullarkat V**, Kirschbaum M, Forman SJ, O'Donnell MR. Reduced-intensity conditioning for allogeneic hematopoietic stem cell transplantation with fludarabine and melphalan is associated with durable disease control in myelodysplastic syndrome. *Bone Marrow Transplant* 2007; 40: 843-850.
7. **Pullarkat V**, Bedell V, Kim Y et al. Neoplastic mast cells in systemic mastocytosis associated with t(8;21) acute myeloid leukemia are derived from the leukemic clone. *Leukemia Research* 2007; 31: 261-265.
8. **Pullarkat V**, Veliz L, Chang K et al. Therapy-Related MLL Translocation Positive Monoblastic Myeloid Sarcoma of the Uterus. *Journal of Clinical Pathology* 2007; 31: 1749-1754.
9. Snyder D, Stein AS, **Pullarkat V** et al. Allogeneic hematopoietic cell transplantation following reduced intensity conditioning for treatment of myelofibrosis. *Biol Bone Marrow Transplant* 2006; 12: 1161-1168.

10. **Pullarkat V**, Lee PP, Scotland R et al. A Phase I trial of SD-9427 (Progenipoeitin) with a multi-peptide vaccine for resected metastatic melanoma. *Clinical Cancer Research* 2003; 9: 1301-1312.
11. **Pullarkat V**, Lau R, Lee SM, Bender J, Weber JS. Large scale monocyte enrichment coupled with a closed bag culture system for generation of human dendritic cells. *Journal of Immunological Methods* 2002; 267: 173-183.
12. **Pullarkat V**, Deo Y, Link J, Spears L, Marty V, Curnow R, Groshen S, Gee C, Weber JS. A phase I study of a HER2/neu bispecific antibody with granulocyte-colony-stimulating factor in patients with metastatic breast cancer that overexpresses HER2/neu. *Cancer Immunology Immunotherapy*. 1999, 48: 9-21.
13. **Pullarkat VA**, Ngo MM, Iqbal S, Espina BM, Liebman HA. Detection of lupus anticoagulant activity identifies patients with autoimmune hemolytic anemia at increased risk for venous thromboembolism. *British Journal of Hematology* 2002; 118: 1166-1169.
14. **Pullarkat V**, Bueso-Ramos C, Lai R et al. Systemic mastocytosis with associated clonal non-mast cell lineage disease: Analysis of clinicopathologic features and activating c-kit mutations. *American Journal of Hematology* 2003; 73: 12-17.
15. Carmel R, **Pullarkat V**. Gene polymorphisms associated with diminished activity of 5,10-methylenetetrahydrofolate reductase do not explain the clinical manifestations of cobalamin deficiency. *British Journal of Hematology* 2003; 120: 907-909.
16. Koya RC, Kasahara N, **Pullarkat V**, Levine AM, Stripecke R. Transduction of acute myeloid leukemia cells with third generation self-inactivating lentiviral vectors expressing CD80 and GM-CSF: effects on proliferation, differentiation and stimulation of allogeneic and autologous antileukemia immune responses. *Leukemia* 2002; 16: 1645-1654.
17. **Pullarkat V**, Bass RD, Feinstein DI, Gong JZ, Brynes RK. Primary autoimmune myelofibrosis: definition of a distinct clinicopathologic syndrome. *American Journal of Hematology* 2003; 72: 8-12.
18. **Pullarkat V**, Pullarkat ST, Calverley DC, Brynes RK. Mast cell disease associated with acute myeloid leukemia: Detection of a new *c-kit* mutation Asp816His. *American Journal of Hematology* . 2000, 65: 307-309.
19. Bass RD, **Pullarkat V**, Feinstein DI, Kaul A, Winberg CD, Brynes RK. Pathology of autoimmune myelofibrosis. *American Journal of Clinical Pathology*, 2001; 116: 211-216.
20. **Pullarkat V**, Kalapura T, Pincus M, Baskharoun R. Oral anticoagulation associated intraspinal hemorrhage. *Archives of Internal Medicine* 2000, 160: 237-240.
21. **Pullarkat V**, Rho H, Collins JM, Liebman HA. Ticlopidine-induced aplastic anemia: Development of chromosomal abnormalities and response to

- immunosuppressive therapy. *American Journal of Hematology* 2000, 63: 141-144.
22. Kostandy G, Katapadi M, **Pullarkat V**, Manzi G, Salama S, Sosler B, Hussain KMA : Skin Metastases: An unusual Presenting Sign of Gastric Carcinoma. *J.Clinical Gastroenterology* 1996,23: 236-237.
 23. Calverley DC, Brass ER, Tsao-Wei DD, **Pullarkat VA**, Espina BM, Hodis HH, Groshen S. A potential role for platelet Fc gamma RIIa in collagen mediated platelet activation associated with atherothrombosis. *Atherosclerosis* 2002; 164: 261-267.
 24. Arzoo K, Sadeghi S, **Pullarkat V**. Pamidronate for bone pain from osteolytic lesions in Langerhans' cell histiocytosis. *N Engl J Med* 2001; 345: 225.
 25. **Pullarkat V**, Medeiros LJ, Brynes RK. Body Cavity based presentation of natural killer cell lymphoma. *Leukemia & Lymphoma* 2005; 46: 293-296.
 26. Lopez F, Parker P, Nademanee A, Rodriguez, Al-Kadhimi, Z. Bhatia R, Cohen S, Falk P, Fung H, Kirschbaum M, Krishnan A, Kogut N, Molina A, Nakamura R, O'Donnell M, Popplewell L, **Pullarkat V**. Rosenthal R, Sahebi F, Smith E, Snyder E, Somlo G, Spielberger R, Stein A, Sweetman R, Zain, J and Forman S. Efficacy of Mycophenolate Mofetil (MMF) in the Treatment of Chronic Graft Versus Host Disease. *Biol BoneMarrowTransplant* 11:307- 313 2005.
 27. Nademanee A, Forman S, Molina A, Fung A, Smith D, Dagis A, Kwok C, Yamauchi D, Anderson A, Falk P, Krishnan A, Kirschbaum M, Kogut N, Nakamura R, O'Donnell M, Parker P, Popplewell L, **Pullarkat V**, Rodriguez R, Sahebi F, Smith E, Snyder D, Stein A, Spielberger R, Zain J, White C, and Raubitschek A. A Phase I/II Trial of High-Dose Yttrium 90 ibritumomab tiuxetan in Combination with High-Dose Etoposide and Cyclophosphamide Followed by Autologous Stem Cell Transplant in Patients with Poor-Risk or Relapsed Non-Hodgkins Lymphoma (NHL) *Blood*, Jul 2005; 10.1182.

Abstracts.

1. **Pullarkat V**, Blanchard S, Tegtmeier B et al. Iron overload adversely affects survival and increases risk of graft-versus-host disease and blood stream infections after allogeneic hematopoietic stem cell transplantation. *Blood* 2007; 110: 2981a.
2. **Pullarkat V**, Bueso-Ramos C, Lai R, Kroft S, Wilson C, Pullarkat S, McCourty A, Lee M, Brynes R. The Asp816His mutation is common in systemic mast cell disease associated with myeloid malignancy. *Blood* 2000; 96: 105a.
3. Viera A, Kasahara N, Cardoso A, **Pullarkat V**, Levine A, Stripecke R. Third generation lentiviral vectors to boost immune response against acute myeloid leukemia. *Blood* 2000; 96: 385b
4. **Pullarkat VA**, Scotland R, Schulz, WE, Chakrabarti D, Weber JS. Lymphoid dendritic cells mobilized with progenipoeitin induce Th1 and peptide-specific cytotoxic T cell responses. *Blood* 2001; 98: 297a

5. **Pullarkat VA**, Ngo MM, Iqbal S, Espina BM, Liebman HA. Detection of lupus anticoagulant activity identifies patients with autoimmune hemolytic anemia at increased risk for venous thromboembolism. *Blood* 2001; 98: 52a.
6. **Pullarkat VA**, Lee S-M, Lau R, Bender JG, Weber JS. Large scale monocyte enrichment with a closed culture system for the generation of dendritic cells. *Blood* 2001; 98: 150b.
7. **Pullarkat VA**, Bass RD, Gong JZ, Feinstein DI, Brynes RK. Primary autoimmune myelofibrosis: definition of a distinct clinicopathologic syndrome. *Blood* 2001; 98: 280b.
8. Wang S, Smith D, Soon PH, Fridey J, Bhatia R, Falk P, Fung H, Kogut N, Krishnan A, Nadamanee A, Nakamura R, O'Donnell M, Parker P, Popplewell L, **Pullarkat V**, Rodriguez R, Sahebi F, Smith E, Snyder D, Spielberger R, Stein A, Tsai NC, Yee D, Zain J, Forman S. Association between stem cell mobilization efficiency and engraftment kinetics among patients with Non-Hodgkin's lymphoma and acute myelogenous leukemia. *Blood* 2003; 102:3573a.
9. Slovak ML, Kopecky KJ, Gundacker H, Appelbaum F, Boldt D, **Pullarkat V**, Forman SJ. Clinical significance of cytogenetic abnormalities in adult acute lymphoblastic leukemia: A Southwest Oncology Group (SWOG) study (S9400). *Blood* 2003; 102: 2223a.
10. Nakamura R, Smith D, Parker P, Senitzer D, Rodriguez R, falk P, Fung H, Kirschbaum M, Kogut N, Krishnan A, O'Donnell M, Popplewell L, **Pullarkat V**, Sahebi F, Smith E, Snyder D, Spielberger R, Stein A, Zain J, Forman SJ Nadamanee A. Impact of transplant CD34+ cell dose on outcomes after allogeneic peripheral blood stem cell transplantation from a matched unrelated donor. *Blood* 2003; 102: 1777a.
11. Kirschbaum M, O'Donnell M, Spielberger R, Bhatia R, **Pullarkat V**, Stein AS et al. Peripheral blood stem cells versus bone marrow for matched sibling transplant in AML and ALL in first remission. *Blood* 2004; 104: 3321
12. Krishnan AY, Chang KL, Fung HC, O'Donnell M, Bhatia R, Spielberger R, **Pullarkat V**, Stein S et al. Impact of allogeneic stem cell transplantation on outcome of biphenotypic acute leukemia. *Blood* 2004; 104: 5144
13. Stein AS, O'Donnell M, Parker P, Spielberger R, Nadamanee A, Bhatia R, Snyder DS, Kirschbaum M, **Pullarkat V**, Kogut N et al. Analysis of Long Term Outcome in Autologous Stem Cell Transplant (ASCT) for Acute Myelogenous Leukemia (AML) in First Remission (CR1) - a 15 Year Experience. *Blood* 2004; 104: 1870.
14. Snyder, DS, Stein, AS, **Pullarkat V** et al. Matched Unrelated Donor Hematopoietic Cell Transplantation Following Reduced-Intensity Conditioning for Treatment of Myelofibrosis. *Blood* 2004; 104: 2769.
15. Sarkodee-Adoo, CB, Stein, AS, O'Donnell, MR, Bhatia, RM, Bolotin, E, Chang, KP, Kirschbaum, M, Kogut, N, Nadamanee, A, Parker, P, **Pullarkat, V**, David Snyder, D, Spielberger, R, Slovak, ML, Alvarnas, J, Fridey, J, Schriber, J, Smith, E, Dagsis, A, Land, J, Palmer, J, Vora, N, and Forman, S. Long Term Survival after ASCT for AML: Multivariate Analysis Shows

Adverse Effect of Collecting and Infusing Larger Numbers of CD 34 Positive Cells.

Blood 2005; 106: 2921.

16. Phase 2 study of targeted intravenous busulfan (IV BU) combined with fractionated total body irradiation (FTBI) and etoposide (VP-16) as preparative regimen for allogeneic peripheral blood stem cell transplant (PBSCT) for patients with poor risk leukemia . Stein AS, O'Donnell MR, Dagis A, Krishnan A, Nademanee A, Nakamura R, Parker PM, Popplewell L, **Pullarkat V**, Rodriguez R, Rosenthal J, Smith EP, Snyder DS, Spielberger R, Synold T, Vora N, Zain J, Sarkodee-Adoo C, Forman SJ. *Biology of Blood and Marrow Transplantation*. February 2006 (Vol. 12, Issue 2 (Supplement 1), 91a
17. Reduced intensity conditioning (RIC) for allogeneic hematopoietic stem cell transplantation (HCT) in patients with AML evolved from MDS or therapy related AML . O'Donnell MR, Roberto R, Stein AS, Palmer J, Slovak M, Nakamura R, Snyder DS, Chang K, **Pullarkat V**, Forman SJ. *Biology of Blood and Marrow Transplantation*. February 2006 (Vol. 12, Issue 2 (Supplement 1), 111a
18. A prospective pilot study of thymoglobulin, cyclosporine (CSA) and MMF as GVHD prophylaxis in unrelated donor (URD) HCT using fludarabine and melphalan (flu/mel) for high-risk patients with hematological malignancies Rodriguez R, Nademanee A, Fang Y, Dagis A, Sahebi F, Parker P, Snyder D, Smith E, Nakamura R, **Pullarkat V**, Senitzer D, Zain J, Stein A, Patane K, Forman S. *Biology of Blood and Marrow Transplantation*. February 2006 (Vol. 12, Issue 2 (Supplement 1),
19. Winston D, van Burik J-A, **Pullarkat V** et al. Prophylaxis against Cytomegalovirus Infections with Oral Maribavir in Allogeneic Stem Cell Transplant Recipients: Results of a Phase 2 Study. (to be presented at the American Society of Hematology Meeting 2006)

Reviews, Book Chapters and Editorials

1. **Pullarkat V**, Forman SJ. Hematopoietic cell transplantation for rare hematologic malignancies. In Thomas' Hematopoietic Cell Transplantation. 4th Edition 2008.
2. **Pullarkat V**, Forman SJ. High dose chemotherapy for breast cancer: Out with the old, In with the new? *Womens Oncology Review* (in press)
3. **Pullarkat V**, Feinstein DI. Medical management of hypersplenism. In *Problems in General Surgery* 2002; 19: 1-8.
4. Stripecke R, Levine AM, **Pullarkat V**, Cardoso AA. Immunotherapy with acute leukemia cells modified into antigen presenting cells: ex vivo culture and gene transfer methods. *Leukemia* 2002; 16: 1974-1983.

Submitted manuscripts

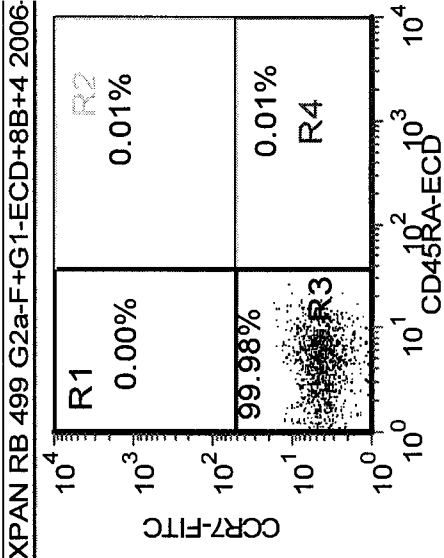
1. **Pullarkat V**, Blanchard S, Tegtmeier B et al. Iron overload adversely affects survival and increases risk of graft-versus-host disease and blood stream infections after allogeneic hematopoietic stem cell transplantation. (Submitted to British Journal of Haematology)
2. **Pullarkat V**, Slovak ML, Dagit A et al. Acute leukemia and myelodysplasia after adjuvant chemotherapy for breast cancer: durable remissions after hematopoietic stem cell transplantation. (Submitted to Journal of Clinical Oncology)
3. Bussel JB, Kuter DJ, **Pullarkat V** et al. Safety and efficacy of long-term treatment with romiplostim in thrombocytopenic patients with chronic ITP. (Submitted to Blood)

T-CELL ANALYSIS
MRN:RB499 (CML-BM)

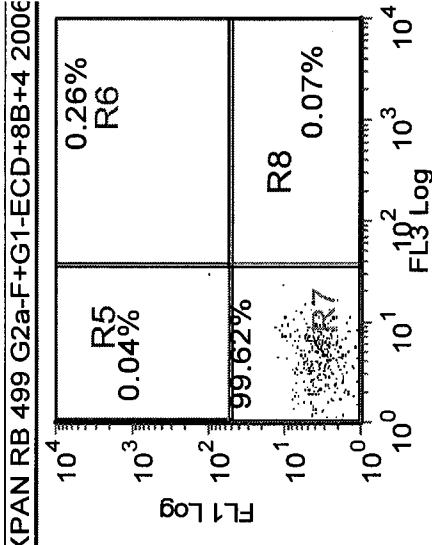
(Pre-expansion)

08/07/06

Supplemental Figure 1



Gated on CD4

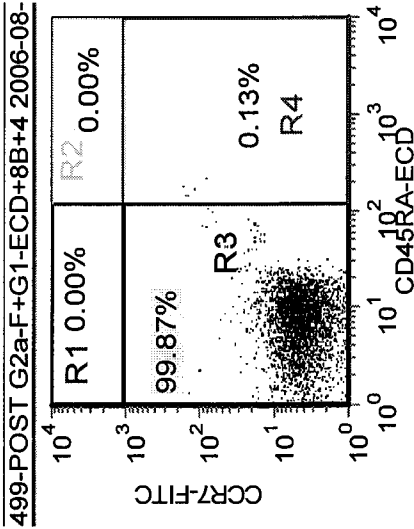


Gated on CD8B

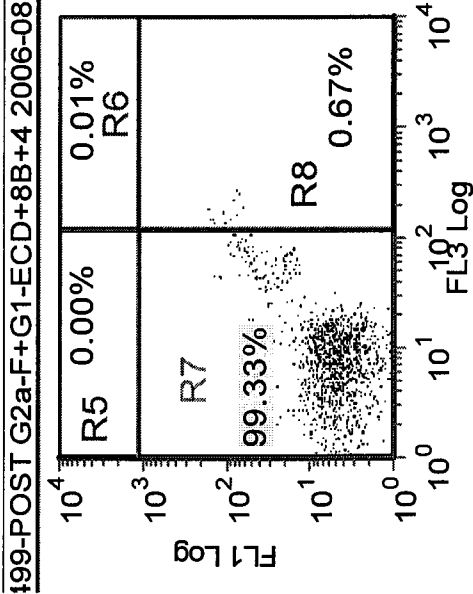
T-CELL ANALYSIS
MRN:RB499 (CML-BM)

(Post-expansion)

08/18/06

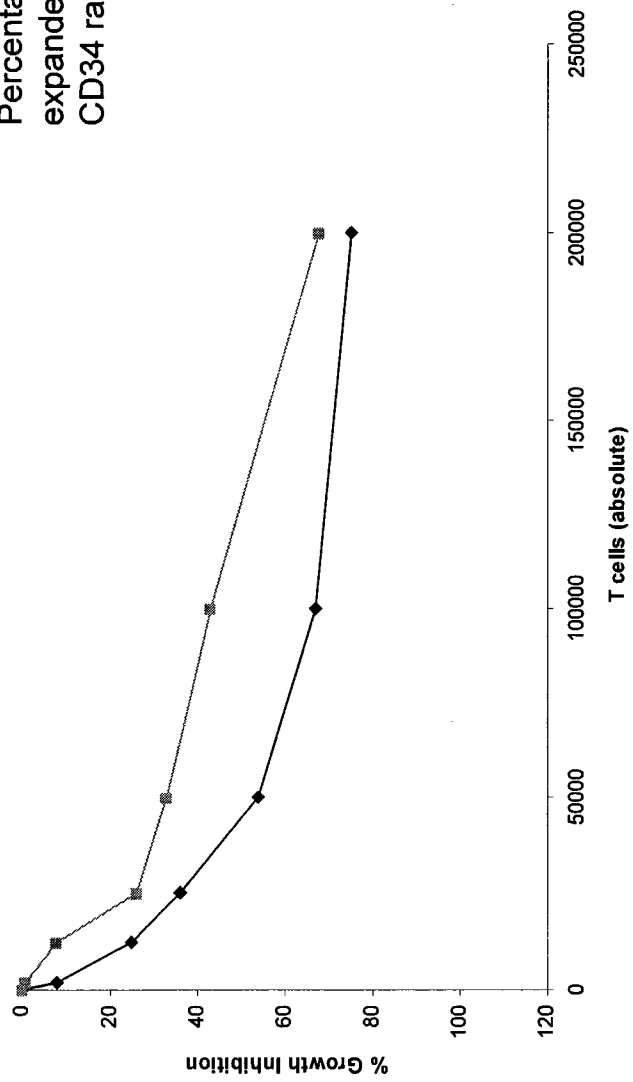


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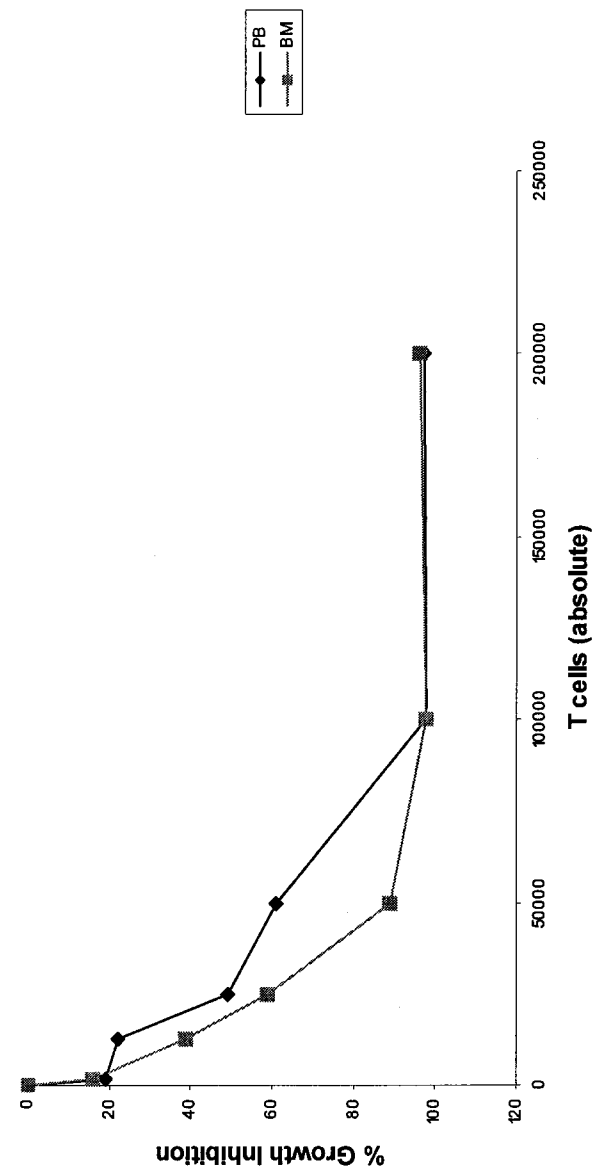


Gated on CD8B

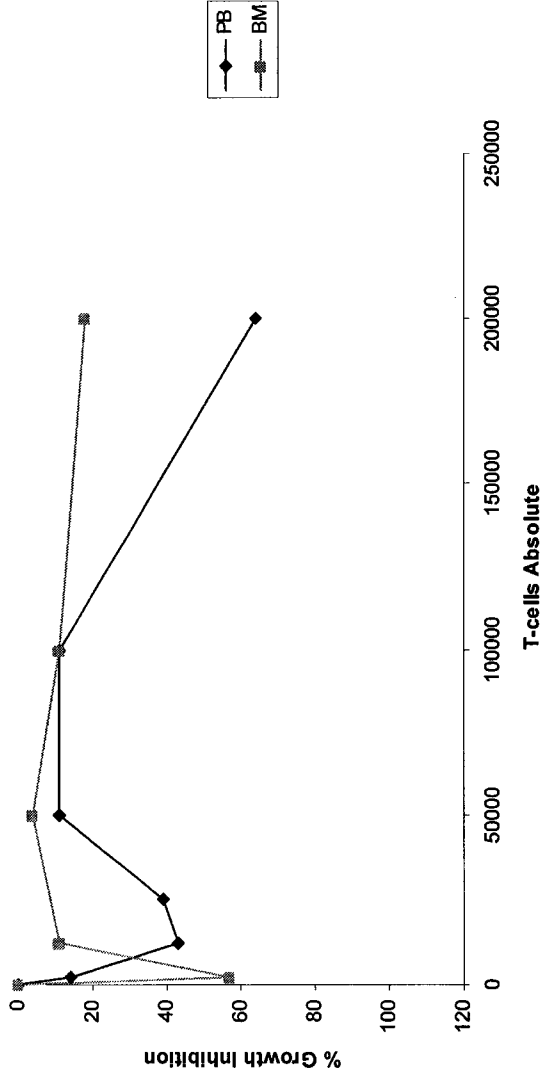
Tcells vs. % Growth Inhibition
(RB210) 8/31/06



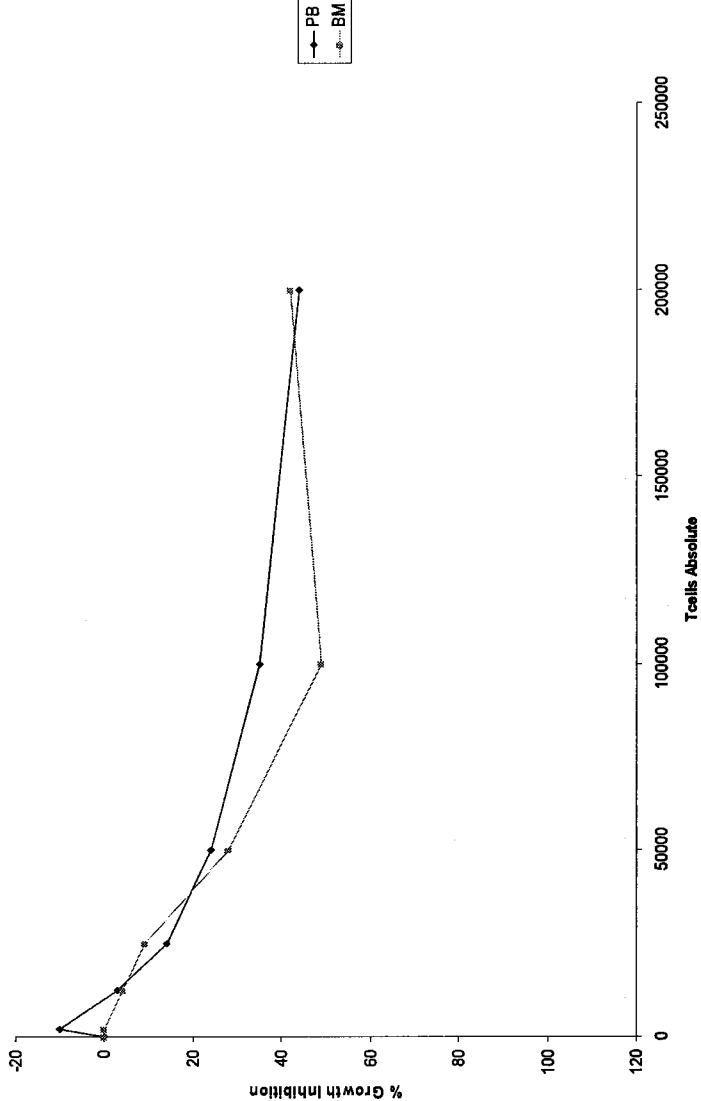
T cells vs. % Growth Inhibition (2nd Patient)



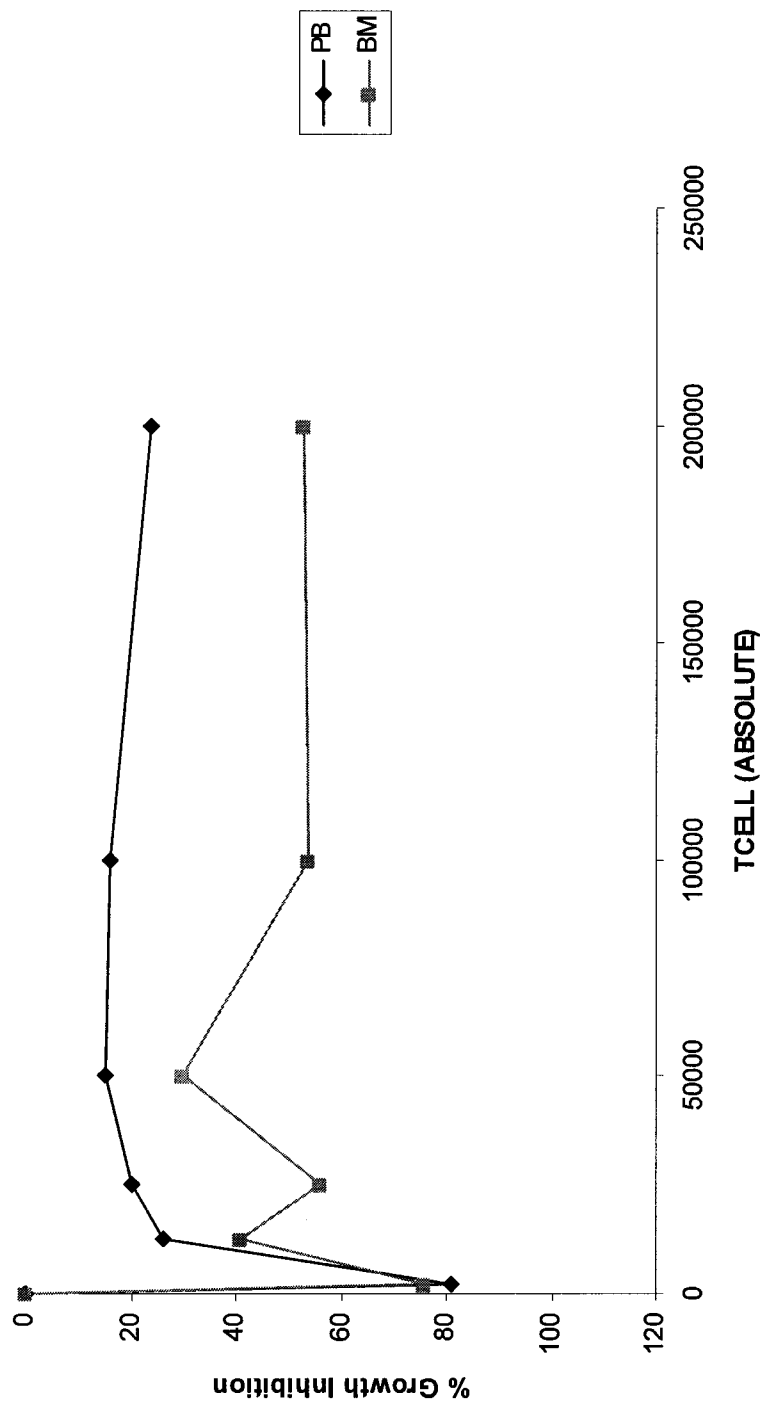
Tcells vs % Growth Inhibition for 3rd patient RB460



Tcells vs % Growth Inhibition for 4th Patient RB 499

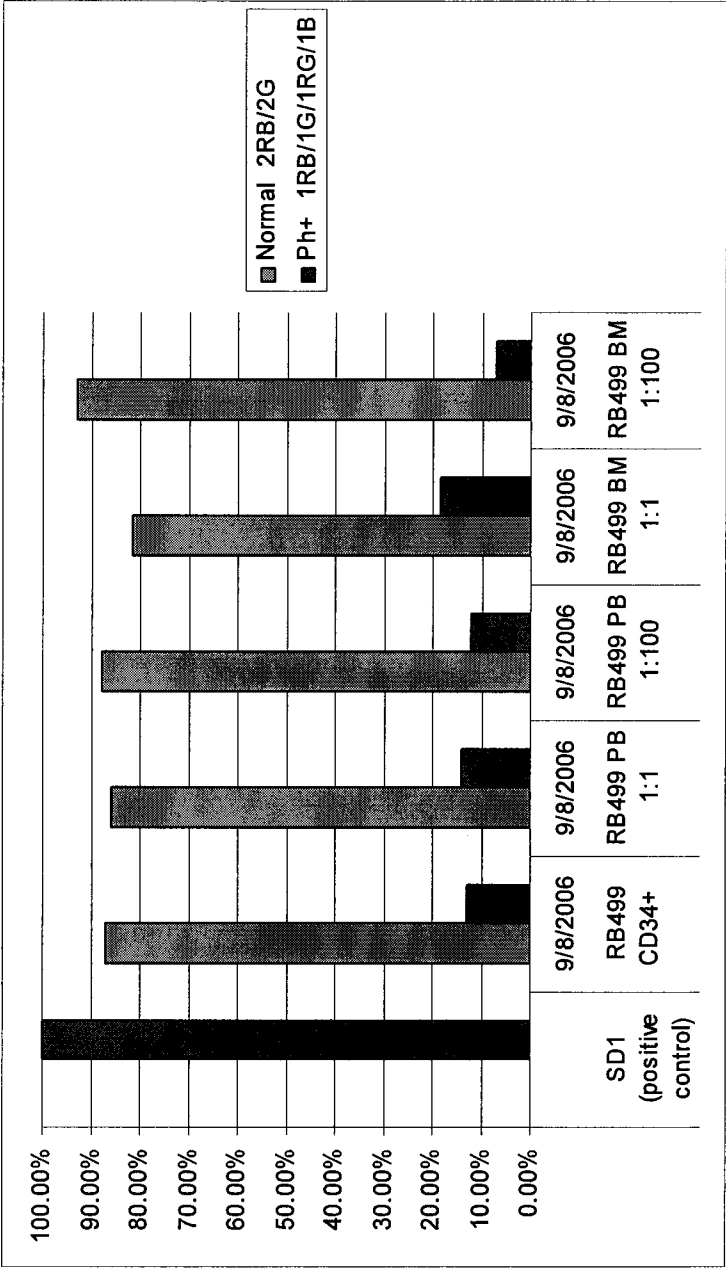


Tcells Vs % Growth Inhibition for 5th patient RB348



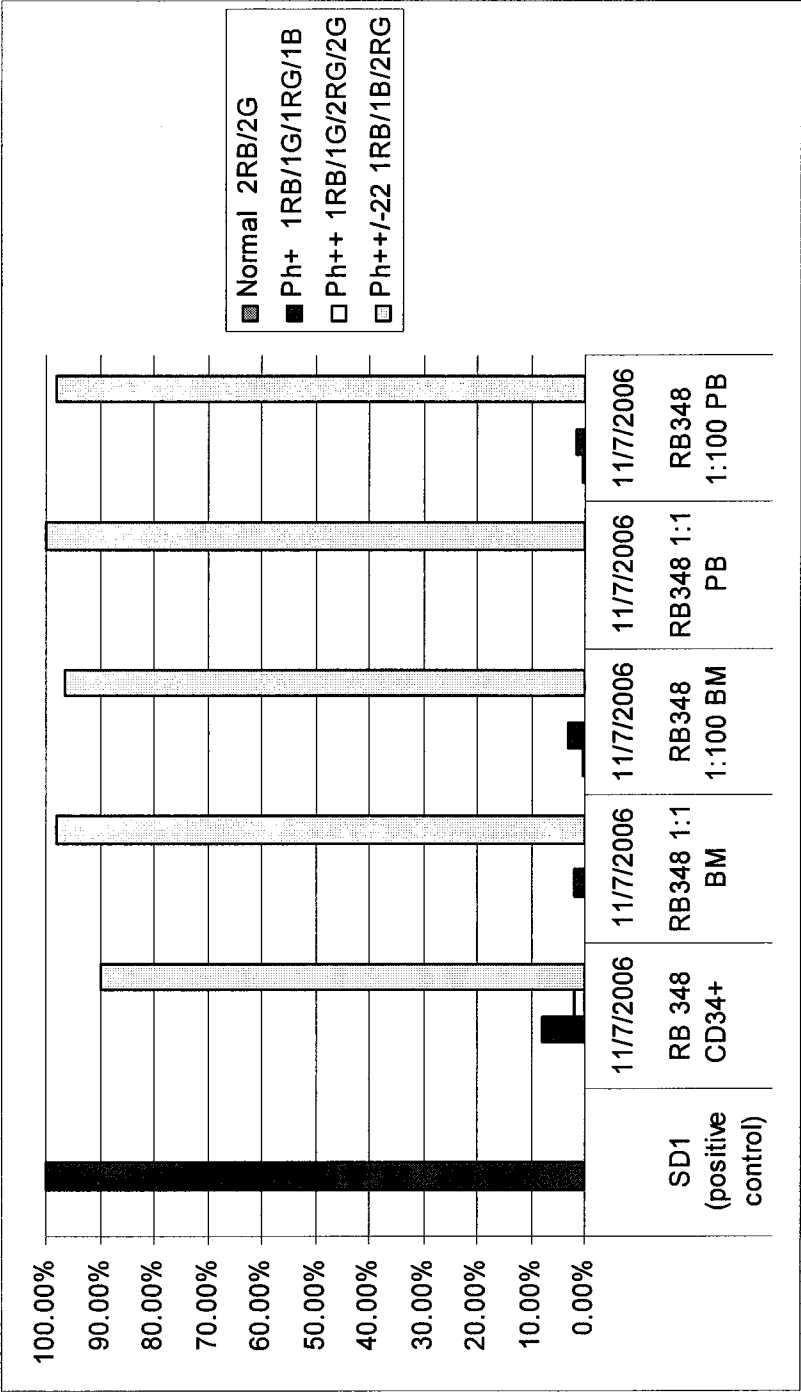
Patient 1

SAMPLE NAME	SAMPLE DATE	Normal 2RB/2G	Ph+ 1RB/1G/1RG/1B	Ph+/-ASS 1RB/1G/1RG	Total Cell Count
SD1 (positive control)			100.00%		200
RB499 CD34+	9/8/2006	87.00%	13.00%		200
RB499 PB 1:1	9/8/2006	86.00%	14.00%		200
RB499 PB 1:100	9/8/2006	88.00%	12.00%		200
RB499 BM 1:1	9/8/2006	81.50%	18.50%		200
RB499 BM 1:100	9/8/2006	93.00%	7.00%		200



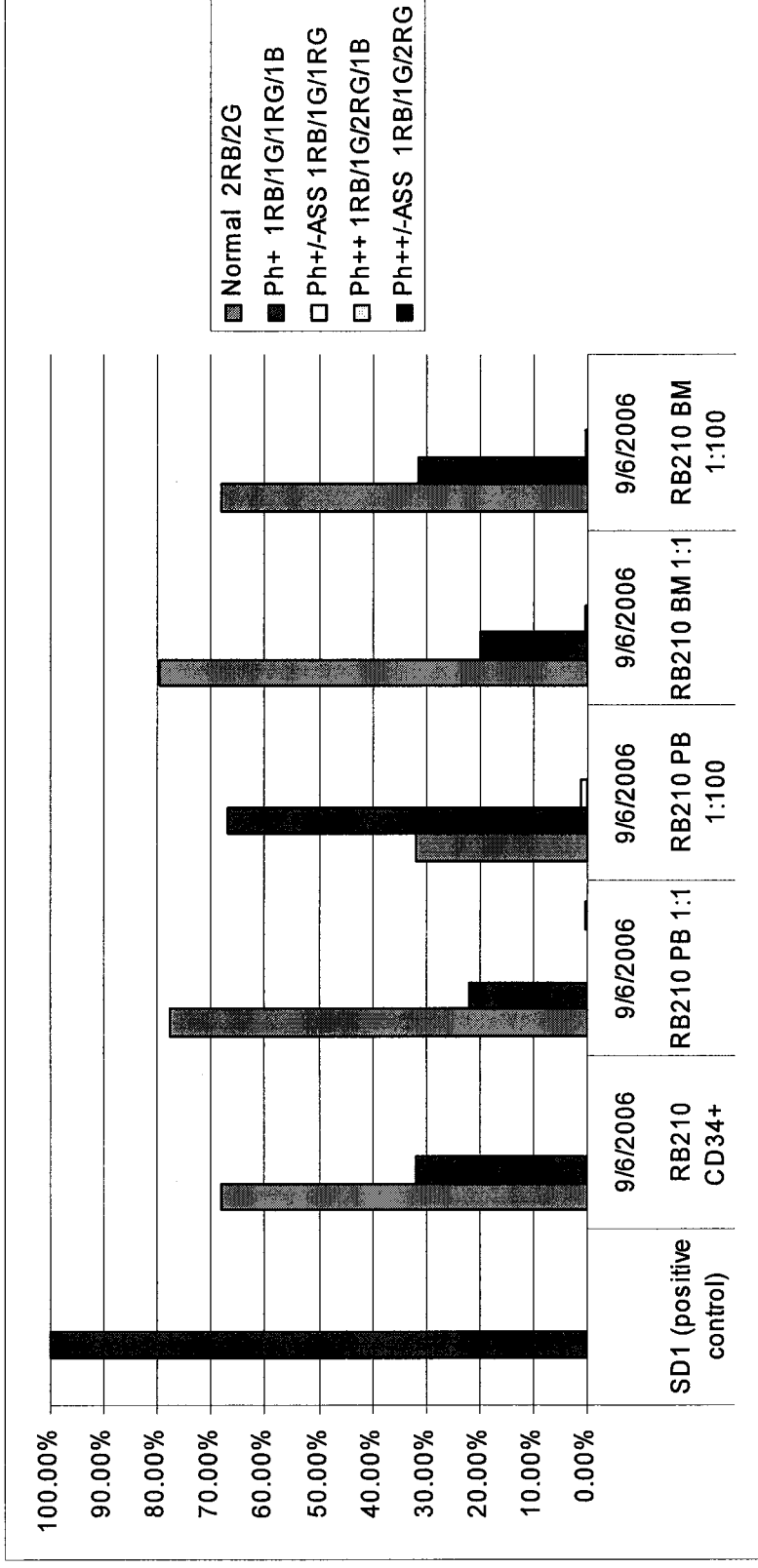
Supplemental Figure 3. Percentages of normal and Ph+ cells in colonies After incubation with expanded M ILs and PBLs at various ratios

SAMPLE NAME	SAMPLE DATE	Normal	Ph+	Ph++	Ph++/-22	Total Cell Count
SD1 (positive control)		2RB/2G	1RB/1G/1RG/1B	1RB/1G/2RG/2G	1RB/1B/2RG	
RB 348 CD34+	11/7/2006		100.00%			200
RB348 1:1 BM	11/7/2006		8.00%	2.00%	90.00%	200
RB348 1:100 BM	11/7/2006	0.50%	2.00%		98.00%	200
RB348 1:1 PB	11/7/2006		3.00%		96.50%	200
RB348 1:100 PB	11/7/2006	0.50%	1.50%		100.00%	200
					98.00%	200



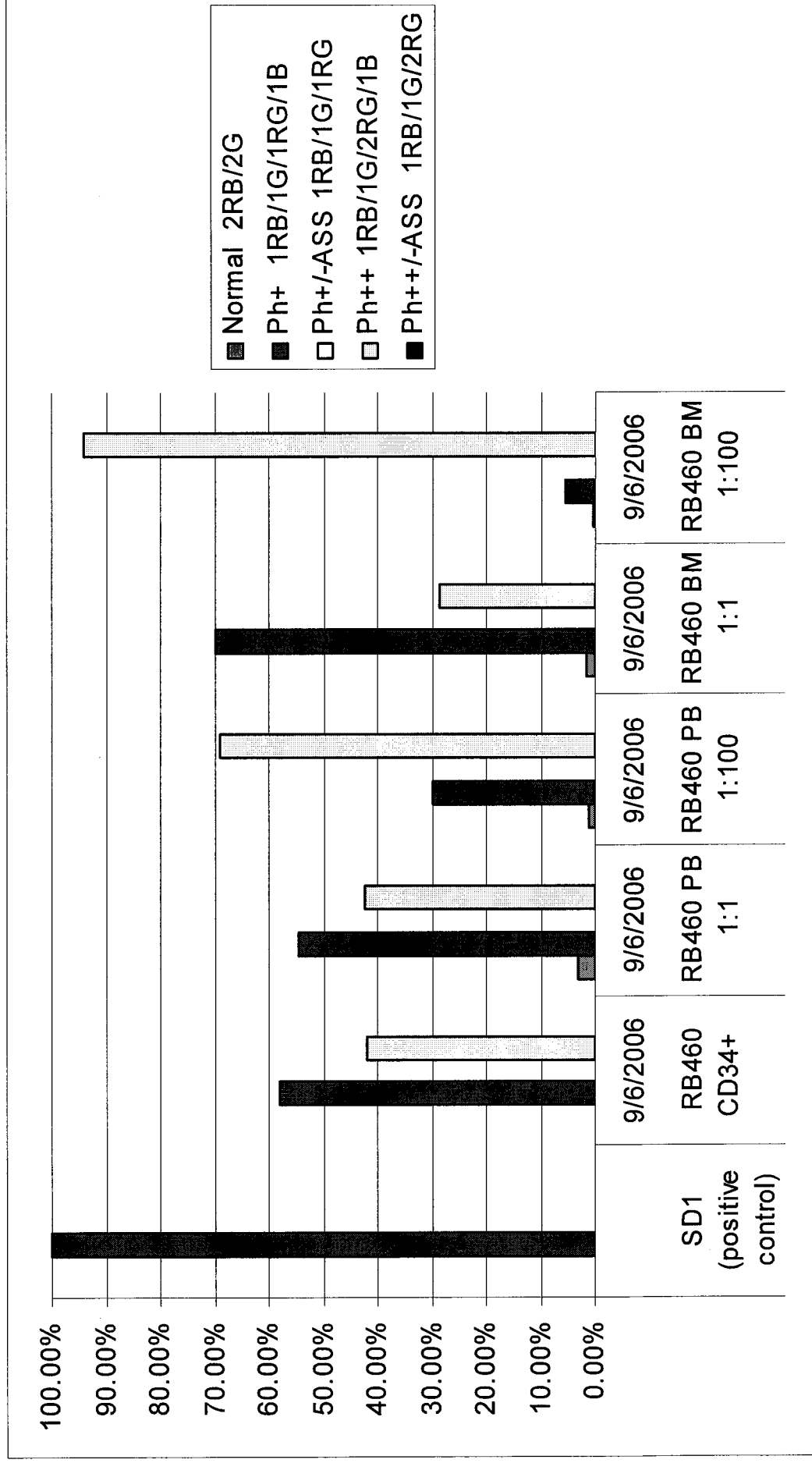
Patient 2

SAMPLE NAME	SAMPLE DATE	Normal 2RB/2G	Ph+ 1RB/1G/1RG/1B	Ph+/-ASS 1RB/1G/1RG	Ph++ 1RB/1G/2RG/1B	Ph++/-ASS 1RB/1G/2RG	Total Cell Count
SD1 (positive control)			100%				200
RB210 CD34+	9/6/2006	68.00%	32.00%				200
RB210 PB 1:1	9/6/2006	77.50%	22.00%			0.50%	200
RB210 PB 1:100	9/6/2006	32.00%	67.00%	1.00%			200
RB210 BM 1:1	9/6/2006	79.50%	20.00%	0.50%			200
RB210 BM 1:100	9/6/2006	68.00%	31.50%	0.50%			200



Patient 3

SAMPLE NAME	SAMPLE DATE	Normal 2RB/2G	Ph+ 3/1G/1RG	Ph+/-ASS 1RB/1G/1RG	Ph++ 1RB/1G/2RG/1B	Ph++/-ASS 1RB/1G/2RG	Total Cell Count
SD1 (positive control)			100%				200
RB460 CD34+	9/6/2006		58.00%		42.00%		200
RB460 PB 1:1	9/6/2006	3.00%	54.50%		42.50%		200
RB460 PB 1:100	9/6/2006	1.00%	30.00%		69.00%		200
RB460 BM 1:1	9/6/2006	1.50%	70.00%		28.50%		200
RB460 BM 1:100	9/6/2006	0.50%	5.50%		94.00%		200



Cell		1st patient	2nd patient	3rd patient	4th patient	5th patient	6th patient
		CML-BM	CML-BM	CML-BM	CML-BM	CML-BM	CML-BM
		8/9/2006	6/21/2006	6/28/2006	8/9/2006		1/14/2008
		PRE	PRE	PRE	PRE	PRE	PRE
	CD3	5.79	67.83	4.27	31.98	4.27	NA
	CD8B	2.52	18.49	0.98	6.72	0.98	6.5
	CD4	1.66	34.8	2.28	23.42	2.28	16.52
	CD56	1.05	22.7	1.59	5.67	1.59	5.99
	CD19	0.94	6.21	0.26	6.61	0.26	26.68
	CD45	7.87	100	5.56	41.45	5.56	NA

		1	2	3	4	5	6
Cell		CML-BM	CML-BM	CML-BM	CML-BM	CML-BM	CML-BM
		8/18/2006	6/22/2006	7/10/2006	8/18/2006		1/29/2008
		POST	POST	POST	POST	POST	POST-CTLA4
	CD3	80.03	91.56	71.32	54.8	77.16	NA
	CD8B	40.87	24.86	6.16	11.1	35.7	2.58
	CD4	23.24	46.47	26.16	38.18	24.87	3.72
	CD56	3.49	1.48	1.26	2.89	1.26	2.98
	CD19	8.52	56.16	0.23	7.31	0.23	0.13
	CD45	60.63	100	77.06	77.37	100	NA

		1	2	3	4	5	6
Cell		CML-PB	CML-PB	CML-PB	CML-PB	CML-PB	CML-PB
		8/9/2006	6/21/2006	6/28/2006	8/9/2006		1/14/2008
		PRE	PRE	PRE	PRE	PRE	PRE
	CD3	11.88	75.13	7.4	51.86	7.4	NA
	CD8B	4.42	22.79	1.56	10.58	1.56	9.26
	CD4	3.75	39.87	4.06	38.53	4.06	45.96
	CD56	2.38	16.53	2.87	13.23	2.87	26.72
	CD19	0.91	5.74	0.33	7.42	0.33	3.48
	CD45	15.93	100	11.62	83.03	11.62	NA

		1	2	3	4	5	6
Cell		CML-PB	CML-PB	CML-PB	CML-PB	CML-PB	CML-PB
		8/18/2006	6/22/2006	7/10/2006	8/18/2006		1/29/2008
		POST	POST	POST	POST	POST	POST-CTLA4
	CD3	74.51	88.9	69.84	59.08	77.16	NA
	CD8B	40.55	25.51	40.68	12.97	35.7	7.1
	CD4	11.9	62.65	12.74	38.18	24.87	7.34
	CD56	7.4	2.85	4.22	7.46	1.35	6.94
	CD19	6.97	74.35	0.15	2.48	27.01	0.54
	CD45	88.07	100	75.08	82.66	100	NA

MEAN	STDEV
22.828	27.78503
6.031667	6.622291
13.49333	13.80709
6.431667	8.261715
6.826667	10.15359
32.088	40.90742

Supplemental Figure 1

Absolute numbers of lymphocyte subsets in bone marrow (CML-BM)

MEAN	STDEV
74.974	13.47014
20.21167	15.99756
27.10667	14.59687
2.226667	1.002989
12.09667	21.91857
83.012	16.92185

MEAN	STDEV
30.734	31.07144
8.361667	8.024548
22.705	20.69022
10.76667	9.887808
3.035	3.029619
44.44	43.42588

MEAN	STDEV
73.898	10.86411
27.085	14.43234
26.28	21.04858
5.036667	2.612299
18.58333	29.12204
89.162	10.91668

and peripheral blood (CML-PB) before (PRE) and after expansion with CD3/CD28 beads

SUB	PRE	1st patient	2nd patient	3rd patient	4th patient	5th patient	6th patient
		BM	BM	BM	BM	BM	BM
	CD4 RA+ 7+	0.00%	0.02%	0.00%	0.01%	0.00%	0.00%
	CD4 RA+ 7-	0.17%	0.11%	0.05%	0.00%	0.05%	39.56%
	CD4 RA- 7-	99.66%	99.86%	99.93%	99.98%	99.93%	60.44%
	CD4 RA- 7+	0.17%	0.01%	0.02%	0.01%	0.02%	0.00%
	CD8 RA+ 7+	0.00%	9.31%	0.00%	0.26%	0.00%	0.00%
	CD8 RA+ 7-	0.15%	0.21%	0.00%	0.04%	0.00%	36.14%
	CD8 RA- 7-	99.85%	32.68%	99.95%	99.62%	99.95%	63.86%
	CD8 RA- 7+	0.00%	57.80%	0.05%	0.07%	0.05%	0.00%

POST	BM	BM	BM	BM	BM	BM
CD4 RA+ 7+	0.00%	0.01%	0.00%	0.00%	0.00%	2.62%
CD4 RA+ 7-	0.00%	0.02%	0.00%	0.00%	0.00%	1.05%
CD4 RA- 7-	99.95%	99.95%	99.95%	99.87%	99.95%	12.04%
CD4 RA- 7+	0.05%	0.02%	0.05%	0.13%	0.05%	84.29%
CD8 RA+ 7+	0.00%	0.01%	0.00%	0.01%	0.00%	0.51%
CD8 RA+ 7-	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
CD8 RA- 7-	99.86%	99.98%	100.00%	99.33%	100.00%	16.33%
CD8 RA- 7+	0.14%	0.01%	0.00%	0.67%	0.00%	83.16%

PRE	PB	PB	PB	PB	PB	PB
CD4 RA+ 7+	0.00%	0.02%	0.00%	0.00%	0.00%	25.15%
CD4 RA+ 7-	0.06%	0.04%	0.00%	0.00%	0.00%	0.27%
CD4 RA- 7-	99.92%	55.68%	100.00%	99.94%	100.00%	5.65%
CD4 RA- 7+	0.02%	44.26%	0.00%	0.05%	0.00%	68.92%
CD8 RA+ 7+	0.04%	0.03%	0.00%	0.05%	0.00%	32.58%
CD8 RA+ 7-	0.06%	0.01%	0.00%	0.00%	0.00%	0.15%
CD8 RA- 7-	99.85%	27.48%	99.14%	99.84%	99.14%	4.79%
CD8 RA- 7+	0.06%	72.48%	0.86%	0.11%	0.86%	62.47%

POST	PB	PB	PB	PB	PB	PB
CD4 RA+ 7+	0.03%	0.00%	0.00%	0.00%	0.00%	2.05%
CD4 RA+ 7-	0.01%	0.00%	0.01%	0.01%	0.01%	0.18%
CD4 RA- 7-	99.89%	99.99%	99.97%	99.87%	99.97%	4.40%
CD4 RA- 7+	0.07%	0.00%	0.02%	0.12%	0.02%	93.37%
CD8 RA+ 7+	0.02%	0.01%	0.00%	0.02%	0.00%	4.12%
CD8 RA+ 7-	0.01%	0.00%	0.00%	0.01%	0.00%	0.00%
CD8 RA- 7-	99.92%	99.99%	100.00%	99.88%	100.00%	4.15%
CD8 RA- 7+	0.05%	0.00%	0.00%	0.10%	0.00%	91.74%

MEAN	STDEV
0.01%	8.3666E-05
6.66%	0.16119382
93.30%	0.16098441
0.04%	0.00064936
1.60%	0.03780993
6.09%	0.14721679
82.65%	0.28398824
9.66%	0.23582888

Supplemental Table 2

Bone marrow (BM) and periphera
blood CD4 and CD8 T cell subsets before (PRE) and
after expansion (POST)

MEAN	STDEV
0.44%	0.01068802
0.18%	0.00427103
85.29%	0.3588259
14.10%	0.34386773
0.09%	0.00206632
0.00%	0
85.92%	0.34091337
14.00%	0.33883961

MEAN	STDEV
4.20%	0.10265815
0.06%	0.00105151
76.87%	0.39127549
18.88%	0.30236809
5.45%	0.13290947
0.04%	0.00060222
71.71%	0.43640682
22.81%	0.34746171

MEAN	STDEV
0.35%	0.00834546
0.04%	0.00070333
84.02%	0.39003255
15.60%	0.38099388
0.70%	0.01677924
0.00%	5.164E-05
83.99%	0.39113483
15.32%	0.37440472